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Review of the doctoral thesis prepared by Ms. Roshanak Zarrin Ghalami entitled "The role of CIA2 and CIL in the regulation of chloroplast biogenesis, photosynthesis and foliar temperature in *Arabidopsis thaliana*"

General remarks

Ms Ghalami's doctoral dissertation was completed at the Department of Plant Genetics, Breeding and Biotechnology of Warsaw University of Life Sciences (SGGW) under the supervision of Prof. Stanisław Karpinski in collaboration with assistant supervisor Dr. Paweł Burdiak. The research was financed by grant from the National Science Center (NCN) OPUS20, No. UMO-2020/39/B/NZ3/02103.

Ms. Ghalami has prepared her doctoral thesis in a hybrid format. The first part is, practically, a condensed version of the PhD thesis. A brief introduction precedes a description of the research objectives and hypotheses. The author outlines the main assumptions of the studies that the transcription factors CIA2 and CIL play a key role in chloroplast development and are indirectly linked to processes that regulate the thermal balance of leaves. She also formulated a general hypothesis suggesting that, under higher irradiance conditions, foliar temperature is determined by the balance between photochemical reactions and non-photochemical quenching (NPQ), as well as evaporative cooling via transpiration.

Ms Ghalami has set out these assumptions in more detail in the form of two research objectives:
1. CIA2 and CIL regulate chloroplast development, including pigment accumulation, thylakoid formation and photosystem II (PSII) assembly.
2. The non-photochemical dissipation of light energy process (NPQ), which depends on the degree of chloroplast maturity, significantly affects foliar temperature.

To carry out the first task, the author selected single and double mutants of the CIA2 and CIL genes for her comprehensive research study. The experimental design involved analysing the seedlings at successive time points during the de-etiolation process. The solution of the second problem required parallel measurements of fluorescence and thermographic parameters.

These sections, *Introduction* and *Research Objectives and Hypotheses*, are only 5 pages long and certainly do not provide a comprehensive information about research topics. Review publication in Cells 15 (2026) 75 and Introduction in publication from Cells 15 (2026) 333 provide a more comprehensive overview of the current understanding of thermoregulation and the regulation of chloroplast biogenesis, however, the author does not refer the reader to these positions. The research objectives and tasks of the thesis are clearly formulated. However, including appropriate references to the articles would emphasise the significance of the research in relation to the phenomenon of thermoregulation and chloroplasts biogenesis. Similarly, the chapter on methodology serves as an introduction to the subjects, in which the author refers to information contained in her publications, although the references could be more precise.

Unlike the previous the chapter *Results* is more detailed and contains most of the results presented in Cells 15 (2026) 333 as well as in bioRxiv manuscript. Furthermore, the figure from the review publication is added as an introduction to the chapters on thermography. The part of data is presented directly in this chapter as a set of figures or tables, whilst for others, only links to the relevant publication are provided. The figures are clear and graphically polished, and the captions are also clear and comprehensive. The statistical analyses of the results are also very well described. *However, the author has not included Figures 5, S5 and S6 or any combination of these figures) from Manuscript No. 3. In my opinion, these figures are crucial for establishing links between the issues under investigation.*

The 'Discussion' chapter is more extensive and covers both main topics of the thesis, but mainly the relationship between thermoregulation and the NPQ phenomenon and stomatal transpiration. The text is divided into subsections that discuss specific phenomena, and it is concluded with a general summary. I would point out that, in discussion, the author refers only a few publications; *and no references to her own data presented in the figures or tables are included. It is a regret that the author did not refer, in this chapter, to her own very good discussion and graphical conclusion about CIA2 and CIL factors from Cells 15 (2026) 333.* Instead, in her doctoral thesis, the author summarised the effects of CIA2 and CIL factors on chloroplast development and thermogenesis in graphical form (from Manuscript No. 3).

The second integral part od doctoral thesis are the attached publications. The first publication (Cells 15 (2026) 75) is a review article discussing plant responses to excess light (absorption of energy in excess, AEE), including short- and long-term adaptive mechanisms and the phenomenon of thermoregulation. According to the authors' statements, the Ms Ghalami contribution to this publication amounts to 70%.
The next publication (Cells 15 (2026) 333) is an experimental study considering genetic, biochemical, structural and photosynthetic analyses of *cia2*, *cil* and *cia2cil* mutants during de-etiolation. Ms Galami's contribution to the listed experiments, analysis of the results and preparation of the manuscript (67%) demonstrate her pivotal role in creating this work.

The PhD thesis also contains the manuscript deposited in the bioRxiv repository. This manuscript describes experiments that investigated the relationship between foliar heat emission, photosynthetic parameters and stomal transpiration. The version included in the doctoral thesis differs from the version available on the bioRxiv server. Among other, it includes a key experiment on the thermoregulation of *cia2cil* mutant and graphical conclusion. The author has not explained these discrepancies. The statement listed the work carried out by Ms Galami. which accounts for 67% of this manuscript.

To summarise my overall assessment of Ms Galami's thesis, I can state that the PhD candidate has clearly establishes her research aims and objectives. The presentation of the research in publications is highly substantive and deserves a mention. However, the structure of the thesis should be clearer; in other words, the PhD student should refer with greater precision to the relevant sections of her publications. The statements clearly indicate the significant contribution of Ms Roshanak Zarrin Galami in research, its analysis and preparation of publications.

Substantive assessment

In her doctoral thesis, the author worked on two research topics that are indirectly linked and come together at the end of the research. The first was to demonstrate the role of transcription factors, CIA2 and CIL, in chloroplast development. The second was to establish a link between changes in foliar temperature and the development of the structure and function of the photosynthetic system. Consequently, these issues require separate comments.

The topic of chloroplast development is being studied at various stages of the process. Ms Galami has focused on the later stages, from 4 hour of light treatment, which include the formation of the thylakoid network, expansion of the grana, and establishment of a fully functional photosynthetic chain.

The author selected *Arabidopsis* plants with mutations in the nuclear transcription factor genes (*cia2*, *cil* and *cia2cil* lines) as a model system for evaluating the process of chloroplast development. Gene expression patterns during the de-etiolation process (4–96 hours) showed that genes involved in chloroplast development (including GLK1, GLK2, HY5, CGA1 and MYBS1) declined moderately in the mutant lines. Furthermore, the expression of genes encoding translocon components and ribosomal proteins decreased in the *cia2cil* line.

Expression of the LHCB2 gene, which encodes the LHCII antenna proteins, and the psbA gene, which encodes the PSII reaction centre, was also reduced in the mutants. In contrast, ELIP1 transcript levels remained largely unchanged between genotypes. The transcription level assay was confirmed by protein content analyses. These experiments showed, particularly in the *cia2cil* line, a slower accumulation of PSII-associated proteins, psbA and Lhcb2, than in the wild type. The author also observed a delay in the synthesis of chlorophyll and carotenoids, as well as in the increase of Photosystem II quantum efficiency and NPQ. The author also noted certain changes in the structure of the thylakoids. However, these microscopic measurements were taken 48 hours after de-etiolation had begun, by which time the thylakoid network had already fully developed.

In this study, Ms Galami conducted a logical series of experiments linking changes in gene expression, protein levels, and the regulation of photosynthetic reactions. It can therefore be concluded that CIA and CILs directly or indirectly influence the expression of genes associated with chloroplast development and that this complex effect delays rather than inhibits the process of chloroplast biogenesis. The author summarised these findings in a schematic diagram, which suggests that CIA2 and CIL play a key role in regulating genes associated with photomorphogenesis.

Agreeing with the authors' general conclusions, I would like to ask about issues related to the structure of the photosynthetic apparatus and NPQ:

(i) *why were the transcripts and protein levels of Lhbc2 measured, even though the Lhcb1 protein is present in greater quantities in Arabidopsis?*

(ii) *Since the NPQ parameter is related to both the PSII and LHCII complexes, did the author consider determining the ratio between them?*

The second topic in the doctoral dissertation is research related to the relationship between NPQ and foliar temperature. Ms Galami used thermographic measurement to analyse changes in foliar temperature under excess light (AEE) with *Arabidopsis*, aspen and oak leaves. The data obtained for background and biological samples indicate that light-induced temperature rises originated within the leaves themselves rather than from environmental heat. The author found that temperature increases differed in time for leaves at different stages of development, as well as for leaves under different intensities of illumination. Furthermore, observed effects seem to be universal for any species.

The author's precise calorimetric measurements indicate that approximately 30% of the light energy absorbed by the leaves under AEE conditions is released as heat. These findings are attributed by the author to metabolic processes taking place within the cell (see Fig. 5). However, it cannot be ruled out that some of the thermal energy emitted is associated with the vibrational energy of the molecules. This may be relevant when comparing the juvenile leaves with fully developed ones, because they differ in their tissue composition. *I would ask you to address this issue, particularly because the author in some of the experiments used white light, i.e. light with a broad spectral range.*

Subsequent experiments were designed to answer the question: How do plants regulate the foliar temperature under high light conditions (AEE)? The authors had been testing two hypotheses: (i) the cooling of leaves is significantly dependent on stomatal transpiration, and (ii) the rise of foliar temperature is mainly associated with dissipation of excess light energy by a non-photochemical quenching (NPQ) mechanism.

To test the first hypothesis, the author blocked the stomatal apparatus with lanolin. Indeed, the foliar temperature increased by about 3°C compared with the control leaves. The relationship between stomatal conductance and changes in foliar temperature has also been confirmed by other studies, for example on the *Arabidopsis tmm-1* mutant.

The second hypothesis is more difficult to prove due to the complex nature of the NPQ mechanism and the difficulty of identifying temperature changes strictly associated with it. In her experiments, the author used different concentrations of DCMU (Q_B-domain inhibitor) to inhibit PSII activity and, consequently, the level of NPQ. She found that an increased concentration of DCMU decreased the values of both NPQ and Fv/Fm, as well as the maximal foliar temperature. Furthermore, it was found that changes to these parameters were linearly correlated with each other.

The author concluded from this research that suppressing NPQ significantly reduces foliar temperature. Furthermore, Ms Ghalami refers to studies involving Arabidopsis with a crk5 kinase mutation, in which a correlation was observed between a decrease in NPQ and cooling of the foliar temperature. Therefore, the author concluded that NPQ is the main cause of internal heat generation in leaves under AEE conditions. Ms Ghalami proposes a broader theory: that NPQ generates internal heat, which is removed by transpiration. Together, these phenomena establish a dynamic thermal balance that depends on both chloroplast physiology and stomatal behaviour. While these findings are quite plausible, they may not be the only factors influencing the leaves' thermal balance.

Nevertheless, I would invite Ms Ghalami to address the following points:

(i) *Is there a risk of leaf damage when using a DCMU spray?*

(ii) *How is the energy absorbed by PSII dissipated, especially under DCMU treatments? Is it plausible that generation of free radicals in endothermic reactions?*

(iii) *Have you considered testing your theory by simultaneously measuring NPQ, stomatal transpiration and foliar temperature in function of increasing light intensity? I think that this experiment would provide a more precise explanation of these correlations.*

The connection of the first with second research tasks involved investigating the thermoregulatory properties of the *cia2cil* mutant. This mutant revealed the reduced pigment content and antenna size as well as smaller thylakoid network. In this case the NPQ and foliar temperature is about 10% lower in comparison to control one. Furthermore, the stomatal conductance in cia2cil leaves is almost two-times higher than control ones, which is probably associated with downregulation of genes related to stomata opening. As Ms Ghalami suggests in her concluding remarks, the CIA2 and CIL factors may affect the overall thermoregulation of leaves indirectly by influencing the synthesis of photosynthetic complexes and the regulation of hormonal balance.

Concluding remarks

Ms Ghalami has conducted highly complex research combining molecular biology, biochemistry, and physiological measurements. The analysis of complex phenomena depends greatly on the type of material used and the limitations of the equipment. Biological material can be highly variable, requiring a sufficiently large number of replicates. Appropriate specialist knowledge is also required to analyse the obtained data. Ms Ghalami has met these requirements, and her excellent analysis of the results is particularly noteworthy.

Ms Ghalami produced several interesting findings in the section describing the *cia2*, *cil* and *cia2cil* lines. She also established a link between leaf thermoregulation and photosynthesis. These results have been discussed in detail, and Ms Ghalami has provided logical interpretations of them. Therefore, it can be concluded that Ms Ghalami presented an original solution to a scientific problem in her doctoral thesis. My comments relate to either the editing of the text or matters of debate. They do not affect my overall positive assessment of the work.

In my opinion, the doctoral thesis presented by Ms. Roshanak Zarrin Ghalami meets the statutory requirements set out in Article 187 of the Act of 20 July 2018, 'Law on Higher Education and Science' (Journal of Laws of 2021, as amended). I am therefore asking the Scientific Council of the Discipline of Biology at Warsaw University of Life Sciences (SGGW) to admit Ms Roshanak Zarrin Ghalami to the subsequent stages of the doctoral degree award procedure.

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